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(54) Title: USE OF INHIBITORS OF DIPEPTIDYL-AMINOPEPTIDASE TO BLOCK ENTRY OF HIV INTO CELLS

(57) Abstract

Inhibitors of Dipeptidyl-Aminopeptidase Type IV having the following general formula: X-Pro-Y-Boropro, where X and Y are chosen from any amino acid (including proline).

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TITLE OF THE INVENTION

Use of Inhibitors of Dipeptidyl-Aminopeptidase to Block Entry of HIV into Cells.

Background of the Invention

This invention relates to inhibitors of the amino peptidase activity of dipeptidyl amino peptidase type IV (DP IV).

DP IV is a serine protease present in many microbes, mammalian cells and tissues, for example, renal tubule cells, intestinal epithelium, and blood plasma. It is also present on the surface of CD-4+ and some CD-8+ T-cells, and in low amounts in the central nervous system. It is thought to be involved in the regulation of the immune response; occurrence of DP IV on a cell surface is associated with the ability of cells to produce interleukin 2 (IL-2). DP IV is also referred to as DAP IV or DPP IV; it is assigned EC number 3.4.14.5.

Three different inhibitors of DP IV are known. One of these is a suicide inhibitor: N-Ala-Pro-O-(nitrobenzyl-)hydroxylamine. (The standard three letter amino acid codes are used in this application; O represents oxygen.) Another is a competitive inhibitor: e-(4-nitro)benzoxycarbonyl-Lys-Pro. The third is a polyclonal rabbit anti-porcine kidney DP IV immunoglobulin.

Summary of the Invention

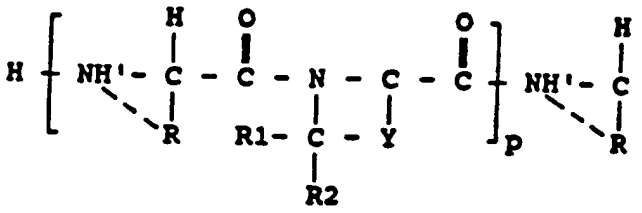
The enzymatic activity of DP IV involves cleaving of a dipeptide from the free amino terminus of a polypeptide. DP IV has a preference for cleaving after a proline, i.e., a proline in the penultimate position from the amino terminus. A free amino terminus is required; thus, DP IV is a postproline cleaving enzyme with a specificity for removing an N-terminal W-Pro dipeptide

from a polypeptide (where W can be any amino acid, including proline). DP IV also will remove a W'-Ala dipeptide from an amino terminus of a polypeptide when W' is an amino acid with a bulky side group, e.g., tyrosine.

This invention concerns potent inhibitors of the enzymatic activity of DP IV. Generally, an α-amino boronic acid analog of proline (boroPro is used to designate one such analog which has the carboxyl group of proline replaced with a $B(OH)_2$ group, where $(OH)_2$ represents two hydrogen groups and B represents boron) is bonded to an amino acid to form a dipeptide with boroPro as the C-terminal residue. These dipeptide prolyl-boronic acids are potent and highly specific inhibitors of DP IV activity and have Ki values in the nanomolar range.

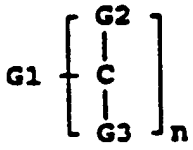
Dipeptides having the boroPro moiety are unstable; thus, inhibitors used in the invention have at least two other amino acids. Generally, the structure of these inhibitors is X-Pro-Y-boroPro where X and Y are chosen from any amino acid (including proline). This tetrapeptide may be lengthened at its N-terminus by addition of one or more dipeptides, each dipeptide having the general formula Z-Pro or Z-ala, where each Z independently is any amino acid (including proline). This general structure is defined in more detail below. These inhibitors function as inhibitors of DP IV because each dipeptide portion is a substrate for DP IV and the final product of the reaction of an inhibitor with DP IV is the dipeptide inhibitor Y-boroPro. The amino terminus of these inhibitors must not be blocked or they lose their inhibitory capacity for DP IV, since DP IV cannot cleave a dipeptide from a blocked N-terminal polypeptide.

Thus, the invention features use of an inhibitory compound having the structure: Group I - Group II. Group I has the structure:

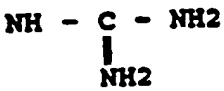


where H represents a hydrogen; C represents a carbon; O represents an oxygen; N represents a nitrogen; each R, independently, is chosen from the group consisting of the R groups of amino acid, including proline; each broken line, independently, represents a bond to an H or a bond to one R group, and each H' represents that bond or a hydrogen; and p is an integer between 0 and 4 inclusive in the preparation of a medicament for administration to a human patient who is infected with HIV, but who is not yet suffering from AIDS, as defined by The Center for Disease Control, Atlanta, Georgia, wherein the compound blocks entry of HIV into uninfected CD26+ cells of the patient by blocking CD26.

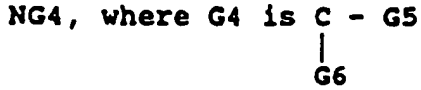
Alternatively, Group I has the structure:



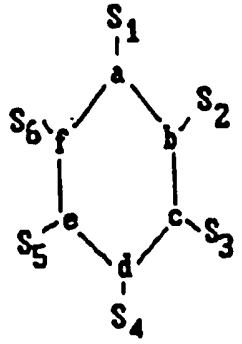
where n is between 0 and 3 inclusive, each G2 and G3 independently is H or C1 - 3 (one to three carbon atoms) alkyl, G1 is NH3 (H3 represents three hydrogens),



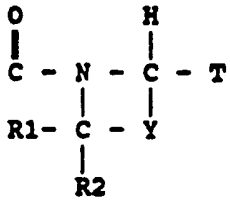
(H2 represents two hydrogens), or



where G5 and G6 can be NH, H, or C1 - 3 alkyl or alkenyl with one or more carbons substituted with a nitrogen. G1 bears a charge, and G1 and Group II do not form a covalently bonded ring structure at pH 7.0. Group I may also have the structure:



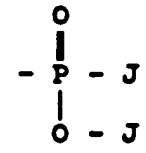
where one or two of the a, b, c, d, e, and f group is N, and the rest are C, and each S1 - S6 independently is H or C1 - C3 alkyl. Group I may also include a five membered unsaturated ring having two nitrogen atoms, e.g., an imidazole ring. Group II has the structure:



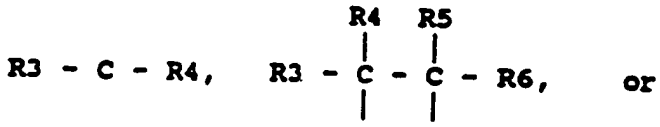
where T is a group of the formula:

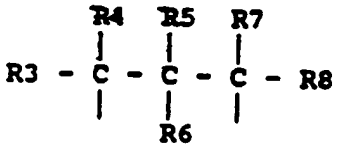
, where each D1 and D2, independently, is a hydroxyl group or a group which is capable of being hydrolysed to a hydroxyl group in aqueous solution at physiological pH; a group of the formula:

where G is either H, fluorine (F) or an alkyl group containing 1 to 20 carbon atoms and optional heteroatoms which can be N, S (sulfur), or O; or a phosphonate group of the formula:



where each J, independently, is O-alkyl, N-alkyl, or alkyl. Each O-alkyl, N-alkyl or alkyl includes 1 - 20 carbon atoms and, optionally, heteroatoms which can be N, S, or O. T is generally able to form a complex with the catalytic site of a DP IV.

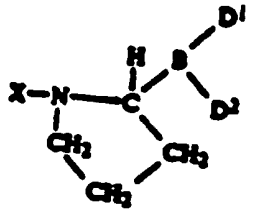
Y is 



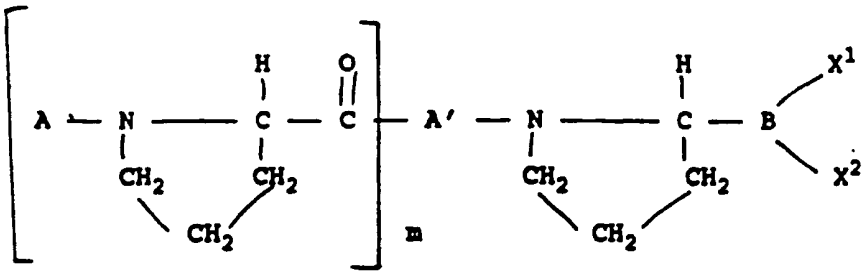
and each R1, R2, R3, R4, R5, R6, R7, and R8, separately is a group which does not significantly interfere with site specific recognition of the inhibitory compound by DP IV, and allows a complex to be formed with DP IV.

In preferred embodiments, T is a boronate group, a phosphonate group or a trifluoroalkyl ketone group; each R1 - R8 is H; each R1 and R2 is H, and each Y is the CH_2-CH_2; each R is independently chosen from the R group of proline and alanine; the inhibitory compound has a binding or dissociation constant to DP IV of at least 10^{-9}M, 10^{-8}M or even 10^{-7}M; the inhibitory compound is admixed with a pharmaceutically acceptable carrier substance; and each D1 and D2, independently, F, or D1 and D2 together are a ring containing 1 to 20 carbon

atoms, and optionally heteroatoms which can be N, S or O. Preferred compounds have the formula



where each D^1 and D^2, independently, is a hydroxyl group or a group which is capable of being hydrolyzed to a hydroxyl group in aqueous solution at physiological pH; and X comprises an amino acid or a peptide which mimics the site of a substrate recognized by a post prolyl cleaving enzyme. It is also preferred that the amino acid derived moieties of the inhibitor be entirely L-isomers, and that the carbon atom bonded to the boron atom also be of the L-configuration. Thus, preferred inhibitors have the structure



wherein m is an integer betwen 0 and 10, inclusive; A and A' are L-amino acid residues such that the A in each repeating bracketed unit can be a different amino acid residue; the C bonded to B is in the L-configuration; the

bonds between A and N, A and C, and between A and N are peptide bonds; and each X^1 and X^2 is, independently, a hydroxyl group or a group capable of being hydrolysed to a hydroxyl group at physiological pH. Synthesis of the pure L-isomers is described in Bachovchin PCT Application US92/09026, hereby incorporated by reference.

The invention is based on the ability of the inhibitors to block CD26 and thus block entry of HIV into CD26-bearing cells. Thus, the inhibitors are administered (preferably orally, in tablet, capsule, or liquid form) to an HIV-infected patient who does not yet exhibit the symptoms of full-blown AIDS, to inhibit entry of virus into healthy, uninfected cells of the patient, in particular, CD4 + lymphocytes. Preferably, the patient has a CD4 + count of at least 200, and more preferably at least 400, at the time of administration of the inhibitor. The inhibitors treat pre-symptomatic HIV-infected patients not by neutralizing virus, as is the case for some AIDS therapies, but by blocking viral entry into the cells.

Unit dosage of the inhibitor is preferably between 10 and 500, more preferably between 20 and 100, and most preferably between 20 and 80 μg/kg body weight, so that several unit doses in pill, tablet, or capsule form, for use in a 68 kg patient will be made available; these unit dose formulations will contain 500, 1500, 5000, and 30,000 μg of inhibitor. These unit dose formulated pills, capsules, or tablets will preferably be in a sustained release form, and will be administered orally on a daily, every other day, or once-per week basis, to provide a daily dosage to the patient in the ranges recited above. Liquid unit dosage formulations for oral or, less preferably, injectable administration can be employed as well.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments, and from the claims.

Description of the Preferred Embodiments

The drawings will first be briefly described.

Drawings

Figure 1 is a diagrammatic representation of several inhibitors; and

Figure 2 is a diagrammatic representation of the synthesis of a boro proline compound.

Structure

The inhibitory compounds useful in the invention have the general structure recited in the Summary of the Invention above. Examples of preferred structures are those referred to as preferred embodiments above.

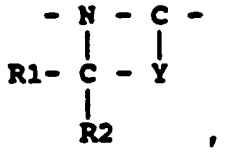
The structure of the inhibitory compounds is such that at least a portion of the amino acid sequence near the cleavage site of a DP IV substrate is duplicated, or nearly duplicated.

The choice of amino acid sequence affects the ability of the inhibitory compound to block CD26. Inhibitory compounds which can complex with DP IV can also block CD26, and thus are useful in the invention. Specificity is determined in a similar fashion, by testing the CD26 blocking effect of a particular inhibitory compound using standard techniques. The inhibitory compounds preferably block CD26 and do not inhibit enzymes necessary for normal cell functions.

The inhibitory compounds include a group (T) which causes the inhibitory compound to complex with DP IV, not only in a competitive fashion, but in a chemically reactive manner to form a strong bond between the inhibitory compound and DP IV. This group thus acts to bind the inhibitory compound to DP IV, and increases the inhibitory binding constant (Ki) of the inhibitory

compound. Examples of such groups include boronates, fluoroalkyl ketones and phosphoramidates (of the formulae given in the Summary above). These groups are covalently bonded to the prolyl residue of the compound, as in the above formula.

The proline or proline analog, represented by



above, is chosen so that it mimics the structure of proline recognized by the active site of DP IV. It can be modified by providing R1 and R2 groups which do not interfere significantly with this recognition, and thus do not significantly affect the Ki of the compound. Thus, one or more hydroxyl groups can be substituted to form hydroxy-proline, and methyl or sugar moieties may be linked to these groups. One skilled in the art will recognize that these groups are not critical in this invention and that a large choice of substitutents are acceptable for R1 and R2.

Synthesis

Synthesis of boroProline

Referring to Figure 1, the starting compound I is prepared essentially by the procedure of Matteson et al., 3 Organometallics 1284, 1984, except that a pinacol ester is substituted for the pinanediol ester. Similar compounds such as boropipecolic acid and 2-azetodine boronic acid can be prepared by making the appropriate selection of starting material to yield the pentyl and propyl analogs of compound I. Further, Cl can be substituted for Br in the formula, and other diol protecting groups can be substituted for pinacol in the formula, e.g., 2,3-butanediol and alpha-pinanediol.

Compound II is prepared by reacting compound I with $[(CH_3)O_3Si]_2N$-Li+. In this reaction hexamethyldisilazane is dissolved in tetrahydrofuran and an equivalent of n-butyllithium added at -78°C. After warming to room temperature (20°C) and cooling to -78°C an equivalent of compound I is added in tetrahydrofuran. The mixture is allowed to slowly come to room temperature and to stir overnight. The alpha-bis[trimethylsilane]-protected amine is isolated by evaporating solvent and adding hexane under anhydrous conditions. Insoluble residue is removed by filtration under a nitrogen blanket, yielding a hexane solution of compound II.

Compound III, the N-trimethysilyl protected form of boroProline is obtained by the thermal cyclization of compound II during the distillation process in which compound II is heated to 100-150°C and distillate is collected which boils 66-62°C at 0.06-0.10 mm pressure.

Compound IV, boroProline-pinacol hydrogen chloride, is obtained by treatment of compound III with HCl:dioxane. Excess HCl and by-products are removed by trituration with ether. The final product is obtained in a high degree of purity by recrystallization from ethyl acetate.

The boroProline esters can also be obtained by treatment of the reaction mixture obtained in the preparation of compound II with anhydrous acid to yield 1-amino-4-bromobutyl boronate pinacol as a salt. Cyclization occurs after neutralizing the salt with base and heating the reaction.

Example 1: Preparation of boroProline-pinacol (H-boroPro-pinacol)

The intermediate, 4-Bromo-1-chlorobutyl boronate pinacol, was prepared by the method in Matteson et al., Organometallics, (3): 1284-1288 (1984), except that conditions were modified for large scale preparations and

the pinacol was substituted for the pinanedoil protecting group.

3-bromopropyl boronate pinacol was prepared by hydrogenboronation of allyl bromide (173 ml, 2.00 moles) with catechol borane (240 ml, 2.00 moles). Catechol borane was added to allyl bromide and the reaction heated for 4 hours at 100°C under a nitrogen atmosphere. The product, 3-bromopropyl bornate catechol (bp 95-102°C, 0.25 mm), was isolated in a yield of 49% by distillation. The catechol ester (124 g, 0.52 moles) was transesterified with pinacol (61.5 g, 0.52 moles) by mixing the component in 50 ml of THF and allowing them to stir for 0.5 hours at 0°C and 0.5 hours at room temperature. Solvent was removed by evaporation and 250 ml of hexane added. Catechol was removed as a crystalline solid. Quantitative removal was achieved by successive dilution to 500 ml and to 1000 ml with hexane and removing crystals at each dilution. Hexane was evaporated and the product distilled to yield 177 g (bp 60 - 64°C, 0.35 mm).

4-Bromo-1-chlorobutyl boronate pinacol was prepared by homologation of the corresponding propyl boronate. Methylene chloride (50.54 ml, 0.713 moles) was dissolved in 500 ml of THF, 1.54 N n-butyllithium in hexane (480 ml, 0.780 moles) was slowly added at -100°C. 3-Bromopropyl boronate pinacol (178 g, 0.713 moles) was dissolved in 500 ml of THG, cooled to the freezing point of the solution, and added to the reaction mixture. Zinc chloride (54.4 g, 0.392 moles) was dissolved in 250 ml of THG, cooled to 0°C, and added to the reaction mixture in several portions. The reaction was allowed to slowly warm to room temperature and to stir overnight. Solvent was evaporated and the residue dissolved in hexane (1 liter) and washed with water (1 liter). Insoluble material was discarded. After drying over anhydrous

magnesium sulfate and filtering, solvent was evaporated. The product was distilled to yield 147 g (bp 110 - 112°C, 0.200 mm).

N-Trimethylsilyl-boroProline pinacol was prepared first by dissolving hexamethyldisilizane (20.0 g, 80.0 mmoles) in 30 ml of THF, cooling the solution to -78°C, and adding 1.62 N n-butyllithium in hexane (49.4 ml, 80.0 mmoles). The solution was allowed to slowly warm to room temperature. It was recooled to -78°C and 4-bromo-1-chlorobutyl boronate pinacol (23.9 g, 80.0 mmoles) added in 20 ml of THF. The mixture was allowed to slowly warm to room temperature and to stir overnight. Solvent was removed by evaporation and dry hexane (400 ml) added to yield a precipitant which was removed by filtration under a nitrogen atmosphere. The filtrate was evaporated and the residue distilled, yielding 19.4 g of the desired product (bp 60 - 62°C, 0.1 - 0.06 mm).

H-boroProline-pinacol.HCl was prepared by cooling N-trimethylsilyl-boroProline-pinacol (16.0 g, 61.7 mmoles) to -78°C and adding 4 N HCL:dioxane (46 ml, 185 mmoles). The mixture was stirred 30 minutes at -78°C and 1 hour at room temperature. Solvent was evaporated and the residue triturated with ether to yield a solid. The crude product was dissolved in chloroform and insoluble material removed by filtration. The solution was evaporated and the product crystallized from ethyl acetate to yield 11.1 g of the desired product (mp 156.5 - 157°C).

Synthesis of boroProline Peptides

General methods of coupling of N-protected peptides and amino acids with suitable side-chain protecting groups to H-boroProline-pinacol are applicable. When needed, side-chain protecting and N-terminal protecting groups can be removed by treatment with anhydrous HCl, HBr, trifluoroacetic acid, or by

catalytic hydrogenation. These procedures are known to those skilled in the art of peptide synthesis.

The mixed anhydride procedure of Anderson et al., J. Am. Chem. Soc., 89:5012 (1984) is preferred for peptide coupling. Referring again to Figure 1, the mixed anhydride of an N-protected amino acid or a peptide varying in length from a dipeptide to tetrapeptide is prepared by dissolving the peptide in tetrahydrofuran and adding one equivalent of N-methylmorpholine. The solution is cooled to -20°C and an equivalent of isobutyl chloroformate is added. After 5 minutes, this mixture and one equivalent of triethylamine (or other sterically hindered base) are added to a solution of H-boroPro-pinacol dissolved in either cold chloroform or tetrahydrofuran.

The reaction mixture is routinely stirred for one hour at -20°C and 1 - 2 hours at room temperature (20°C). Solvent is removed by evaporation, and the residue is dissolved in ethyl acetate. The organic solution is washed with 0.20 N hydrochloric acid, 5% aqueous sodium bicarbonate, and saturated aqueous sodium chloride. The organic phase is dried over anhydrous sodium sulfate, filtered, and evaporated. Products are purified by either silica gel chromatography or gel permeation chromatography using Sephadex™ LH-20 and methanol as a solvent.

Previous studies have shown that the pinacol protecting group can be removed in situ by preincubation in phosphate buffer prior to running biological experiments; Kettner et al., J. Biol. Chem. 259: 15106-15114 (1984). Several other methods are also applicable for removing pinacol groups from peptides including boroProline and characterizing the final product. First, the peptide can be treated with diethanolamine to yield the corresponding diethanolamine boronic acid ester,

which can be readily hydrolyzed by treatment with aqueous acid or a sulfonic acid substituted polystyrene resin as described in Kettner et al., id. Both pinacol and pinanediol protecting groups can be removed by treating with BC13 in methylene chloride as described by Kinder et al., J. Med. Chem., 28: 1917. Finally, the free boronic acid can be converted to the difluoroboron derivative (-BF2) by treatment with aqueous HF as described by Kinder et al., id.

Similarly, different ester groups can be introduced by reacting the free boronic acid with various di-hydroxy compounds (for example, those containing heteroatoms such as S or N) in an inert solvent.

Example 2: H-Ala-boroPro

Boc-Ala-boroPro was prepared by mixed anhydride coupling of the N-Boc-protected alanine and H-boroPro prepared as described above. H-Ala-boroPro was prepared by removal of the Boc protecting group at 0°C in 3.5 molar excess of 4 N HC1-dioxane. The coupling and deblocking reactions were performed by standard chemical reaction. Ala-boroPro has a Ki for DP IV of -1 x 10^{-9}M. Boc-blocked Ala-boroPro has no affinity for DP IV.

The two diastereomers of H-Ala-boroPro-pinacol can be partially separated by silica gel chromatography with 20% methanol in ethyl acetate as eluant. The early fraction appears by NMR analysis to be 95% enriched in one isomer. Because this fraction has more inhibitory power against DP IV than later fractions (at equal concentrations) it is probably enriched in the L-boroPro isomer.

One significant drawback with H-Ala-boroPro as an inhibitor for DP IV is that it decomposes in aqueous solution at neutral pH and room temperature (20-25°C) with a half-life of around 0.5 hour. Many dipeptide derivatives with a free N terminal amino group and a

functional group (such as a difluoromethyl ketone) on the C-terminus are similarly unstable due to intramolecular reaction. A six member ring is formed between the amino and C-terminal functional groups and undergoes subsequent further reaction, such as hydrolysis. DP IV bound inhibitor is more stable, consistent with the hypothesis that decomposition is due to an intramolecular reaction.

H-Pro-boroPro is more stable than H-Ala-boroPro. The Ki of H-Pro-boroPro for DP IV is about 1 x 10^{-8}M, and it decomposes in aqueous solution at room temperature (20 - 25°C) with a half life of about 1.5 hours. Although the affinity of H-Pro-boroPro is about 10-fold less than that of H-Ala-boroPro, the increased stability is advantageous.

Because of the relatively short half life of the above dipeptides inhibitory compounds of the invention are formed as tetrapeptides or longer peptides as shown in the general formula above. These inhibitory compounds are substrates for DP IV yielding the dipeptide inhibitor W-boroPro. These tetrapeptide boronic acids are generally stable and can be administered by any standard procedure to act as a substrate for DP IV and then as a source of a potent DP IV inhibitor. The advantages of such tetrapeptides is that inhibitor is released only in the vicinity of active DP IV. These tetrapeptide boronic acids can be made by the method of mixed anhydride coupling by one of ordinary skill in the art, e.g., Mattason, Organametallics 3:1284 to 1288, 1984.

Test Systems

The following are examples of systems by which the inhibitory activity of the above described inhibitory compounds can be tested on DP IV. As an example H-Ala-boroPro is used to test each of these systems. Inhibitory compounds can be tested by simply substituting them for H-Ala-boroPro.

DP IV is purified from pig kidney cortex by the method of Barth et al., Acta Biol. Med. Germ. (1974) 32:157, and Wolf et al., Acta Biol. Med. Germ. (1978) 37:409, and from human placenta by the method of Puschel et al., E. Eur. J. Biochem. (1982) 126:359. H-Ala-boroPro inhibits both enzymes with a Ki of -1.0×10^{-9}M.

Human Peripheral Blood Mononuclear Cells

H-Ala-boroPro was tested for its influence on PHA-induced proliferation of human peripheral blood mononuclear cells. Human peripheral blood mono-nuclear cells were obtained from healthy human donors by Ficoll-Hypaque density gradient centrifugation. The cells are washed three times in RPMI 1640 medium and resuspended to a concentration of a 1×10^6 in RPMI. 10% human serum was used as necessary.

The proliferative response of lymphocytes was measured using 3H-Thymidine incorporation. MNC cells [Ford, W.L. in Handbook of Experimental Immunology edit. by.: D.M. Weir. Blackwell Scientific Publications, Oxford, 1978. p. 23.6] (5×10^3) were distributed into wells of round-bottom microtitre plates (Nunc) and incubated in the presence or absence of various dilutions of antigen, mitogen, lymphokine or other agent of interest. Cells were cultured in an atmosphere of 5% CO_2 in air for 72 hours after which ^{3}H-Thymidine (0.5 uCl/well; 2.0 Ci/mM sp.act., New England Nuclear) was added 6 hours before termination of culture. The cells were harvested with a multiple automatic harvester, and ^{3}H-thymidine incorporation assessed by liquid scintillation counting. ^{3}H thymidine incorporation was determined relative to control values in the absence of inhibitor. Inhibitor was added to give a final concentration of 1×10^{-4}M, but lower concentrations can be used.

Other Embodiments

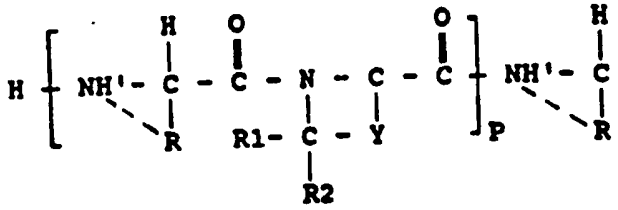
Other embodiments are within the following claims. For example, other inhibitors can be created which mimic the structure of Ala-boroPro. Examples of such inhibitors are shown in Fig. 2 and include Ala-boroPro. These inhibitors generally have a boroPro group, or its equivalent, described above in the Summary of the Invention, and a positively charged amine group. The inhibitors are designed so that minimal interaction of the amine and boroPro groups occurs, and thus no cyclic structure is formed a pH 7.0. These inhibitors interact and/or bind with DPIV.

What is claimed is:

1. Use of compound having the structure

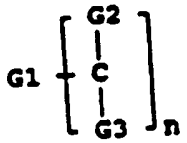
Group I - Group II

where Group I has the structure:



wherein each R, independently, is chosen from the group consisting of the R groups of an amino acid including proline; each broken line, independently, represents a bond to an H or a bond to one said R group, and each H' represents said bond or a hydrogen; p is an integer between 0 and 4 inclusive;

or Group I has the structure:



where n is between 0 and 3 inclusive,

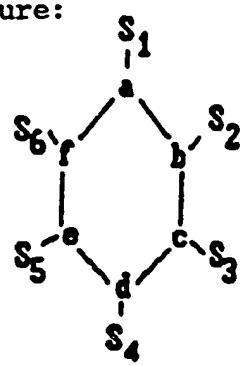
each G2 and G3 independently is H or C1 - 3 alkyl,

G1 is NH3, NH - C(NH2) - NH2, or

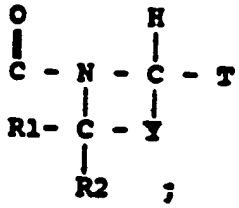
NG4, where G4 is C(G6) - G5

where G5 and G6 can be NH, H, or C1 - 3 alkyl or alkenyl with one or more carbons substituted with a nitrogen; provided that G1 bears a charge and G1 and Group II do not form a covalently bonded ring structure at pH 7.0;

or Group I has the structure:



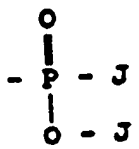
where one or two of said a, b, c, d, e, and f is N and the rest are C, and each S1 - S6 independently is H or C1 - C3 alkyl; where Group II has the structure:



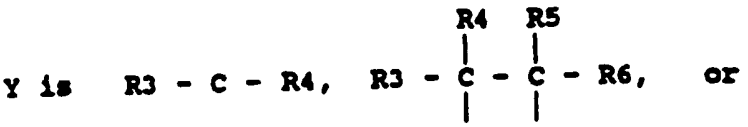
T is a group of the formula:

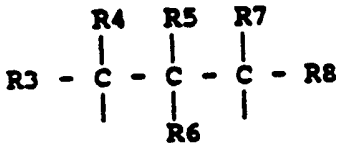
- B- D1, where B is boron and each D1 and D2, independently, is a hydroxyl group or a group which is capable of being hydrolysed to a hydroxyl group in aqueous solution at physiological pH; a group of the formula:

where G is either H, F or an alkyl group containing 1 to 20 carbon atoms and optional heteroatoms which can be N, S, or O; or a phosphonate group of the formula:



where each J, independently, is O-alkyl, N-alkyl, or alkyl, each said O-alkyl, N-alkyl or alkyl comprising 1 - 20 carbon atoms and, optionally, heteroatoms which can be N, S, or O; said T being able to form a complex with the catalytic site of a dipeptidyl-aminopeptidase type IV (DP IV) enzyme;

Y is 



and each R1, R2, R3, R4, R5, R6, R7, and R8, separately is a group which does not significantly interfere with site specific recognition of said inhibitory compound by said DP IV, and allows said complex to be formed with said DP IV for the preparation of a medicament for the treatment of a patient infected with HIV but not suffering from AIDS, wherein said compound blocks entry of HIV into uninfected CD26+ cells of said patient by blocking CD26.

2. The use of claim 1, wherein T is a boronate group.

3. The use of claim 1, wherein T is a phosphonate group or a trifluoroalkyl ketone group.

4. The use of claim 1 wherein each R1 - R8 is H.

5. The use of claim 1 or 2 wherein each R1 and R2 are H, and each Y is CH_2 - CH_2.

6. The use of claim 5 wherein each R is independently chosen from the R group of proline and alanine.

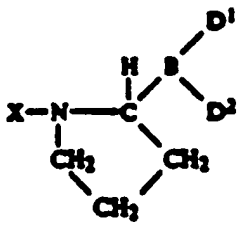
7. The use of claim 1, wherein said compound has a binding or dissociation constant to said DP IV of at least 10^{-9}M.

8. The use of claim 1, wherein said compound has a binding constant to said DP IV of at least 10^{-8}M.

9. The use of claim 1 admixed within a pharmaceutically acceptable carrier substance.

10. The use of claim 1 wherein each D1 and D2 is, independently, F or D1 and D2 together are a ring containing 1 to about 20 carbon atoms, and optionally heteroatoms which can be N, S, or O.

11. The use of claim 1 wherein said compound has the formula

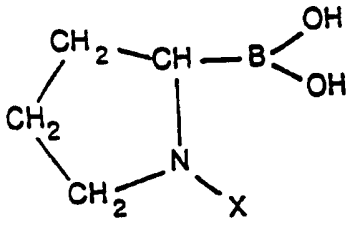


where each D^1 and D^2, independently, is a hydroxyl group or a group which is capable of being hydrolysed to a hydroxyl group in aqueous solution at physiological pH;
and X comprises an amino acid or a peptide which mimics the site of a substrate recognized by a post prolyl cleaving enzyme.

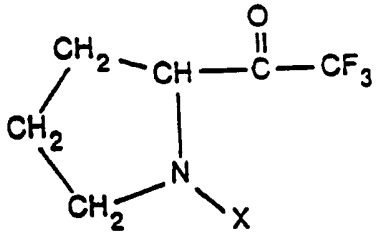
12. The use of claim 1 wherein said medicament is a unit dosage form of said compound formulated for oral administration.

13. The use of claim 12 wherein said unit dosage form is a pill, capsule, or tablet.

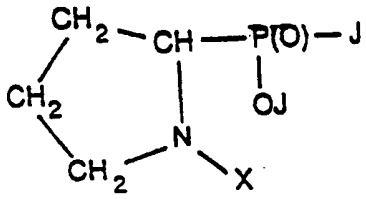
14. The use of claim 12 wherein said unit dosage form contains 500-30,000 μg of said compound.



Prolyl Boronate

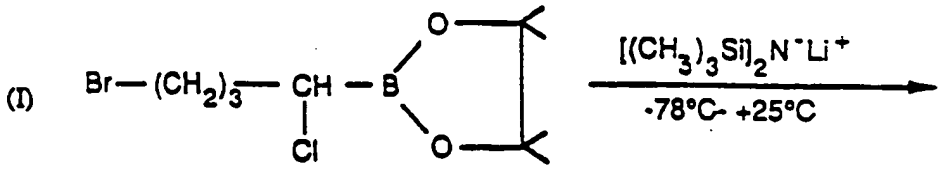


Prolyl Trifluoro alkyl ketone

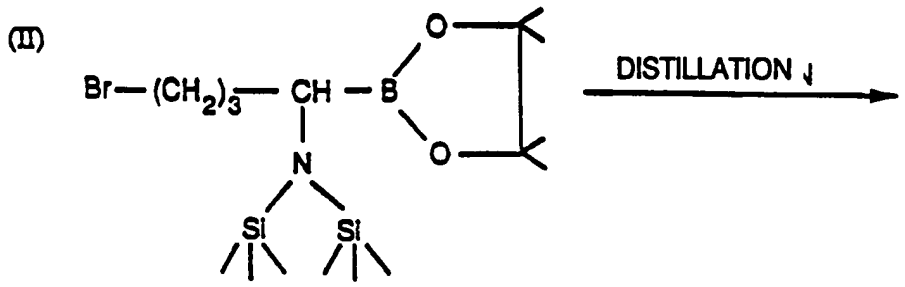


Prolyl phosphonate

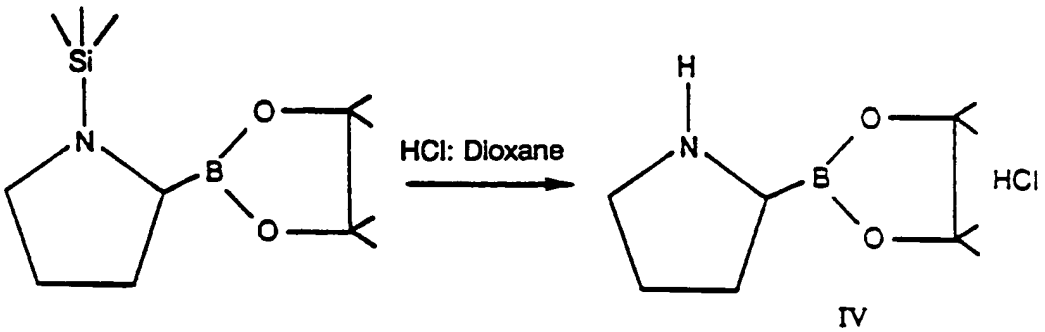
FIG. 1



4-bromo-1-chlorobutyl boronate pinacol

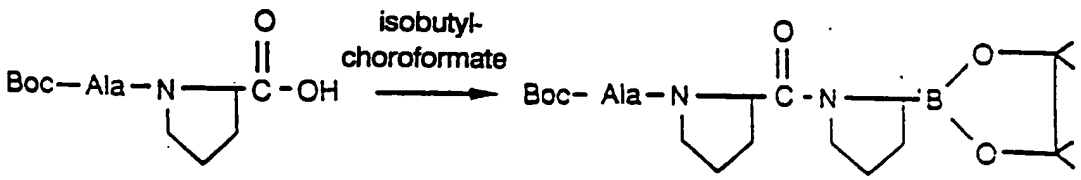


4-bromo-1[(bistrimethylsilyi) amino] butyl boronate pinacol



1-trimethylsilyi-boroProline pinacol
(III)

IV
boroProline-pinacol-HCl



IV +

Boc-Ala-Pro boroPro-Pinacol

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10423

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/00
US CL :514/18
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/18; 530/331.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS Online: structure search.
APS: boronic ester, peptide, dipeptidyl aminopeptidase.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,069,123 (Shenvi et al.) 12 February 1985, see entire document.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 14 January 1994	Date of mailing of the international search report 26 JAN 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer DAVID LUKTON Telephone No. (703) 308-0196